CANADIAN JOURNAL OF RESEARCH

VOLUME 23 FEBRUARY, 1945 NUMBER 1

— SECTION E —

MEDICAL SCIENCES

Contents

Page

Bio-Assay of Vitamin C in Rose Hips—*J. Tuba, M. M. Carter, and G. Hunter* - - - - - - - - - - - 1

Approximate Nutrient Composition of Dried Rose Hips—*J. Tuba, G. Hunter, and J. Kastelic* - - - - - - 5

Distribution of Riboflavin in Fresh and Processed Fish—*W. S. Hoar and M. Barberie* - - - - - - - - - - 8

NATIONAL RESEARCH COUNCIL
OTTAWA, CANADA

CANADIAN JOURNAL OF RESEARCH

The *Canadian Journal of Research* is issued in six sections, as follows:

A. Physical Sciences	D. Zoological Sciences
B. Chemical Sciences	E. Medical Sciences
C. Botanical Sciences	F. Technology

For the present, each of these sections is to be issued six times annually, under separate cover, with separate pagination.

The *Canadian Journal of Research* is published by the National Research Council of Canada under authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research. The *Canadian Journal of Research* is edited by a joint Editorial Board consisting of members of the National Research Council of Canada and the Royal Society of Canada.

EDITORIAL BOARD

Representing National Research Council	*Representing* Royal Society of Canada	
Dr. R. Newton (*Chairman*) President, University of Alberta, Edmonton.	Dr. C. C. Coffin, Professor of Chemistry, Dalhousie University, Halifax.	Section III
Dr. J. B. Collip, Director, Research Institute of Endocrinology, McGill University, Montreal.	Prof. J. K. Robertson, Department of Physics, Queen's University, Kingston.	
Dr. J. A. Gray, Professor of Physics, Queen's University, Kingston.	Prof. J. R. Dymond, Royal Ontario Museum of Zoology, Toronto.	Section V
Dr. O. Maass, Professor of Physical Chemistry, McGill University, Montreal.	Dr. C. L. Huskins, Professor of Genetics, McGill University, Montreal.	

Ex officio, Dr. W. H. Cook, Editor-in-Chief,
Director, Division of Applied Biology,
National Research Laboratories, Ottawa.

Editorial Committee

Editor-in-Chief, Dr. W. H. Cook
Editor Section A, Prof. J. K. Robertson
Editor Section B, Dr. C. C. Coffin
Editor Section C, Dr. C. L. Huskins
Editor Section D, Prof. J. R. Dymond
Editor Section E, Dr. J. B. Collip
Editor Section F, Dr. E. L. Harrington

Manuscripts should be addressed:

Editor-in-Chief,
Canadian Journal of Research,
National Research Council, Ottawa, Canada.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 23, SEC. E. FEBRUARY, 1945 NUMBER 1

BIO-ASSAY OF VITAMIN C IN ROSE HIPS[1]

BY J. TUBA[2], M. M. CANTOR[3], AND G. HUNTER[4]

Abstract

Rose hips, as a dry powder to ensure uniformity of sampling, have been assayed for vitamin C, guinea pigs being used as the test animal. It is here proved by the curative growth method of Harris and Olliver and by the serum phosphatase method of Gould and Schwachman, that the values given for ascorbic acid by titration with 2, 6-dichlorophenolindophenol in rose hips are substantially correct.

Introduction

Rose hips are by far the richest known natural source of vitamin C. The ripe, wet flesh or hypanthium of native Alberta roses contains from 1300 to 3500 mg. % ascorbic acid (5). These estimates were obtained by means of titrations with 2,6-dichlorophenolindophenol. Although Harris and Olliver find excellent agreement between biological and chemical assays on fruits and vegetables if the titration with the dye is done rapidly in acid solution, it is well known that substances other than ascorbic acid reduce the dye and interfere with the determination. In addition, the dye method does not estimate the dehydroascorbic acid, which is physiologically active. The very high ascorbic acid values determined by chemical assay on rose hips may thus be questioned and accordingly biological assays are here recorded. These were carried out with dry rose hip powder, using the curative growth method of Harris and Olliver as well as the more recent Gould and Schwachman curative method based on the estimation of serum alkaline phosphatase.

Experimental

Curative Growth Method

Three series of experiments were carried out using the method of Harris and Olliver (2), but substituting an adapted basal diet the mixed components of which were first heated at 100° C. for four hours to destroy any vitamin C that might be present. In addition to the basal diet, consisting of 45% bran, 25% crushed oats, and 30% skim milk powder, the animals received cod liver oil and a salt mixture in the standard manner.

[1] *Manuscript received September 5, 1944.*
Contribution from the Department of Biochemistry, University of Alberta, Edmonton, Alta.

[2] *Lecturer in Biochemistry.*
[3] *Associate Professor of Biochemistry.*
[4] *Professor of Biochemistry.*

Series I and II consisted of young male guinea pigs that weighed around 200 gm. when they were started on the basal diet plus a daily supplement for each animal of 15 gm. of fresh cabbage. The guinea pigs were weighed daily and, as each animal attained a weight just over 300 gm., the cabbage was withdrawn. The animals were allowed to remain on this scorbutic diet until a sudden drop in weight occurred in the negative controls or until just before the loss in weight occurred in the others. Harris and Olliver allowed 10 days as the average for this period of depletion and provided graded supplements on the 11th day. We repeatedly found this period to vary from 16 to 22 days and selected the 16th day as the one on which to begin feeding the supplements. Iwanoff and Bukin (3) suggest that the Harris method may be speeded up by starting the period of depletion when the animals weigh between 250 and 275 gm. In Series III we made use of such animals but did not find a shortening of the period of depletion.

Series I, the negative control experiment, consisted of a group of six animals; Series II consisted of two groups of six animals. In one group each animal received a daily supplement of 0.3 mgm. pure *l*-ascorbic acid; in the other group each animal was given daily an amount of rose hip powder equivalent to 0.3 mgm. ascorbic acid as determined by dye titration. This amount according to Harris and Olliver is insufficient to maintain a constant weight. The 12 animals in Series III were treated in a similar manner except that each received a supplement of 0.5 mgm. ascorbic acid or an amount of rose hip powder equivalent to it. This level of supplement is sufficient for maintenance of constant weight. We planned a fourth series at a supplemental level of 0.7 mgm. to permit normal growth but could not proceed with the experiment because of a scarcity of animals.

Results

The average weight curves of guinea pigs receiving 0.0, 0.3, and 0.5 mgm. ascorbic acid daily and of those receiving the rose hip equivalent of the two latter values are shown in Fig. 1. The curves in each instance show excellent agreement between the standard and the unknown. On the basis of this evidence it is clear that the reduction of 2, 6-dichlorophenolindophenol by dry rose hip powder is due to ascorbic acid.

Curative Method Based on the Estimation of Serum Alkaline Phosphatase

Gould and Schwachman (1) have shown that serum phosphatase in the guinea pigs is of osteoblastic origin and that in scurvy, where there is impaired osteoblastic activity, the serum alkaline phosphatase level is markedly decreased. They have further demonstrated that, following the administration of ascorbic acid in daily doses of more than 0.25 mgm., osteoblastic activity recovered and was associated with a concomitant rise in the phosphatase level. Their method of assay (1) is based on these criteria.

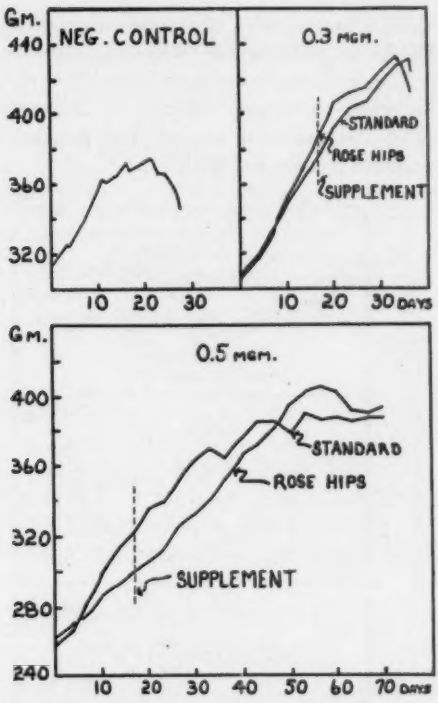

FIG. 1. *Average weight curves of guinea pigs, fed 0, 0.3, and 0.5 mgm. ascorbic acid, and dried powdered rose hips equivalent to 0.3 and 0.5 mgm. as determined by dye titration. Six animals per group.*

Six young male guinea pigs weighing between 250 and 275 gm. were selected for this assay. Blood samples (0.5 ml.) were obtained by heart puncture, and the serum alkaline phosphatase estimated by the method of Shinowara *et al.* (4) as modified by Gould and Schwachman. The animals were then placed on the scorbutic diet and divided into three groups. The animals in Group I were bled on days 0, 16, and 22. Between days 16 and 22, one animal was given an oral supplement of 0.25 mgm. pure *l*-ascorbic acid daily, while its mate received an equivalent amount as rose hip powder. The two animals in Group II were bled on days 0, 16, 18, 23, and 25. Between days 18 and 25 a daily supplement of 0.5 mgm. ascorbic acid was received by one animal and an equivalent amount in rose hip powder by the other. The two animals in Group III were bled on days 0, 17, and 23. In this instance the supplements were 0.7 mgm., administered between days 17 and 23.

Results

The curves representing the concentration of serum alkaline phosphatase in the three groups are shown in Fig. 2. The marked fall in phosphatase level

was associated in each instance with the appearance of scorbutic symptoms. The phosphatase level rose and recovery was apparent when the supplements were provided. Animals on ascorbic acid supplements and on equivalent amounts of rose hip powder behaved precisely in the same manner. This is further evidence that the material in rose hip powder that reduces 2, 6-dichlorophenolindophenol is ascorbic acid.

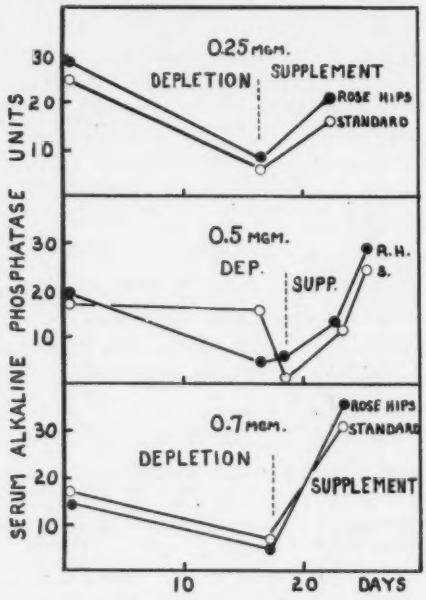

FIG. 2. *The serum alkaline phosphatase of guinea pigs fed a scorbutogenic diet supplemented with 0.25, 0.5, and 0.7 mgm. ascorbic acid and rose hip powder containing equivalent amounts of ascorbic acid as determined chemically.*

Conclusion

Bio-assay evidence is presented to show that the reduction of 2,6-dichlorophenolindophenol by dry rose hip powder is due to ascorbic acid.

References

1. GOULD, B. S. and SCHWACHMAN, H. J. Biol. Chem. 151 : 439-543. 1943.
2. HARRIS, L. J. and OLLIVER, M. Biochem. J. 36 : 155-182. 1942.
3. IWANOFF, N. N. and BUKIN, V. N. Abstract, Chron. Botan. 6 : 204-207. 1941.
4. SHINOWARA, G. Y., JONES, L. M., and REINHART, H. L. J. Biol. Chem. 142 : 921-933. 1942.
5. TUBA, J., HUNTER, G., HUTCHINSON, M. J., and KENNEDY, L. L. Can. J. Research, C, 21 : 363-373. 1943.

APPROXIMATE NUTRIENT COMPOSITION OF DRIED ROSE HIPS[1]

By Jules Tuba[2], George Hunter[3], and Joseph Kastelic[4]

Abstract

The dried fleshy part of the fruit of the wild rose, besides being the richest known source of vitamin C, is also a rich source of vitamin A, and in addition contains appreciable amounts of thiamin, riboflavin, and niacin.

The material contains about 30% fermentable sugar—a mixture of about equal parts of glucose and fructose. An additional 10% of the same sugars is set free by hydrolysis.

The protein content is about 5.5%, and lipids, 1.5%.

Introduction

The very high concentration of ascorbic acid in rose hips seemed to us to justify at least a cursory examination of other likely nutrients. The following analytical summary, with references to methods indicated in parentheses, of our observed average values for some different constituents is based on the dried matter of ripe hips from which the achenes had been removed. Dry matter amounts to about 35% of the ripe hypanthium, or fleshy part of the fruit of the wild rose.

Results and Discussion

Analytical Summary

Constituent	Concentration percent
Ash	5.6 gm.
Protein	5.45 gm.
Fats (ether extract)	1.58 gm.
Carbohydrates	
(*a*) Dry matter hydrolysed for 30 min. with 0.2 *N* H_2SO_4	
Substances reducing alkaline copper (3)	36.3 gm.
Non-fermentable reducing substances (1)	6.5 gm.
Fermentable sugars (1)	29.8 gm.
Fructose (11)	12.9 gm.
Glucose (11)	16.9 gm.
(*b*) In unhydrolysed aqueous extract	
Substances reducing alkaline copper	26.2 gm.
Non-fermentable reducing substances	6.2 gm.
Fermentable sugars	20.0 gm.
Fructose	9.5 gm.
Glucose	10.5 gm.

[1] *Manuscript received September 25, 1944.*

Contribution from the Department of Biochemistry and Department of Field Crops, University of Alberta, Edmonton.

[2] *Lecturer in Biochemistry.*

[3] *Professor of Biochemistry.*

[4] *Research Assistant.*

ANALYTICAL SUMMARY—*Concluded*

Constituent	Concentration percent
Vitamins	
Ascorbic acid (9)	5000 mgm.
Carotene (2)	7500 I.U. vitamin A
Thiamin (5)	0.34 mgm.
Riboflavin (6)	0.295 mgm.
Niacin (7)	3.13 mgm.

Tests for starches and dextrins were negative.

Analyses for ash, protein, fats, and carbohydrates were all done on the same preparation of dried, powdered flesh of *Rosa acicularis*. The vitamin assays, on the other hand, were done on various dried preparations.

Our studies on the vitamin C content of Alberta rose hips have already been reported (9). The ascorbic acid values, which are among the highest reported anywhere, have been found in hips of native roses to be 1300 to 3500 mgm. % in the wet flesh or hypanthium, and, on the average, about 5000 mgm.% on a dry basis.

For the hips of Scandinavian roses, Svensson (8) has found carotene equivalent to 6000 to 10,000 I.U. of vitamin A per 100 gm. Wokes *et al.* (10) report carotene equivalent to 6000 I.U. of vitamin A per 100 gm. of dried extracts of rose hips and conclude that British roses contain as much vitamin A as carrots. We have found carotene equivalent to about 2500 I.U. vitamin A per 100 gm. of wet flesh on the basis of spectrographic analysis (2), although colorimetric values, which do not distinguish carotene from certain other plant pigments, give nearly 10,000 I.U. On a dry basis the carotene equivalent in the ripe hypanthium of rose hips is 7500 I.U. of vitamin A, which is a very high value for a fruit.

Wokes *et al.* (10) and Pyke (4), who used the thiochrome method, report that there is no thiamin in rose hips. Using the same methods we were not only unable to find any of the vitamin but failed to recover added thiamin. This is not surprising in view of the fact that the presence of appreciable amounts of ascorbic acid interferes with the thiochrome estimation.

The values for those members of the B group of vitamins that we estimated in rose hips are among the higher ones recorded for fruits.

We have not made any attempt to estimate vitamin P in rose hips, but according to Wokes and his co-workers, there are considerable amounts of this vitamin present.

References

1. ARCHBOLD, H. K. Ann. Botany, 2 : 183-202. 1938.
2. PETT, L. B. and LEPAGE, G. A. J. Biol. Chem. 132 : 585-593. 1940.
3. PLANK, J. E. VAN DER. Biochem. J. 30 : 457-483. 1936.
4. PYKE, M. Biochem. J. 34 : 330-334. 1940.
5. SCHULTZ, A. S., ATKIN, L., and FREY, C. N. Ind. Eng. Chem. Anal. Ed. 14 : 35-39. 1942.

6. SNELL, E. E. and STRONG, F. M. Ind. Eng. Chem. Anal. Ed. 11 : 346-350. 1939.
7. SNELL, E. E. and WRIGHT, L. D. J. Biol. Chem. 139 : 675-686. 1941.
8. SVENSSON, E. Skand. Arch. Physiol. 73 : 237-254. 1936.
9. TUBA, J., HUNTER, G., HUTCHINSON, M. J., and KENNEDY, L. L. Can. J. Research, C, 21 : 363-373. 1943.
10. WOKES, F., JOHNSON, E. H., ORGAN, J. G., and JACOBY, F. C. Nature, 151 : 279. 1943.
11. YEMM, E. W. Proc. Roy. Soc. (London), B, 117 : 483-504. 1935.

DISTRIBUTION OF RIBOFLAVIN IN FRESH AND PROCESSED FISH[1]

By William S. Hoar[2] and Marjorie Barberie[3]

Abstract

The riboflavin content of 125 different samples of fish was determined microbiologically. The reliability of the microbiological method for use on fish was established. Fish muscle varies greatly in its riboflavin content but, in general, contains less than meat. Skin, roe, and liver have a higher content than muscle but much lower content than the glandular organs from mammals. Molluscan tissues contain more than mammalian muscle but much less than mammalian glands. There is no relationship between the size of the fish and its riboflavin content. Freezing and smoking do not cause appreciable losses of riboflavin. Canning, salting, and drying may produce great losses.

In recent years, reliable riboflavin values have been published for a number of foods—notably cereals and meats. There have, however, been few figures published for fish. Many of the values available for fish may be questioned because of the unreliability and variability of the older methods of determination. The present method of choice is the microbiological method of Snell and Strong (7). This has been used for fish and fish products in only a few instances (3, 5). The number of assays performed has been small and in no case was the reliability of the microbiological method tested for the fish products. Further, there are no figures for the riboflavin content of fish of the Canadian Atlantic Coast. In this investigation we have studied the microbiological method as applied to the determination of riboflavin in fish, and have obtained what appear to be reliable figures for the distribution of the vitamin in fishes of the Atlantic Coast of Canada.

Materials

Canned and processed fish were purchased locally or contributed by the New Brunswick canners*. Fresh fish were obtained locally and from the Fisheries Research Board of Canada**. Samples fast frozen in Halifax were shipped to Fredericton in insulated containers. They were kept in this condition until analysed—usually within 48 hr. Fish shipped in ice were received for analysis within 12 hr. Lobster and shell fish were obtained alive.

[1] *Manuscript received August 21, 1944.*

Contribution from the Biology Department, University of New Brunswick, Fredericton, N.B., with financial assistance from the National Research Council of Canada.

[2] *Professor of Zoology.*

[3] *Research Assistant.*

* *Connors Bros., Black's Harbour; and Fred Magee, Port Elgin.*

** *Courtesy of Dr. S. A. Beatty, Halifax, N.S.; and Dr. A. W. H. Needler, St. Andrews, N.B.*

Methods

Sampling

In dealing with the canned products, the entire content of a can was passed through a meat chopper, mixed thoroughly, and a suitable amount taken for assay.

Muscle, skin, and liver of the fresh fish samples were assayed separately. If the fish were large, the entire skin or muscle was taken from one side of the body; if small, the entire skin or muscle from one fish or several fish was used. In any case the sample was ground in a meat chopper, mixed thoroughly, and a suitable amount taken for assay. In dealing with the glands, entire organs from the large fish or organs from several small fish were treated in a similar manner. The entire shell content—meat and fluid—from a number of the fresh molluscan specimens were combined and a suitable amount taken for assay.

Moisture Determination

Moisture was determined in duplicate on all samples assayed. Suitable amounts of the ground material—2 to 3 gm.—were dried to constant weight in a Fisher Isotemp oven at 95° to 100° C.

Extraction of Riboflavin

Several procedures will liberate riboflavin from tissues. In our studies difficulties were encountered in securing clear extracts owing to the high fat content of certain forms—e.g. mackerel, sardines—and certain organs, such as liver and skin. Since fatty substances have been shown to stimulate the growth of the organism it was necessary to remove these. In outline the method adopted is an acid hydrolysis, followed by a precipitation of protein. This precipitation carries with it most of the fatty materials.

The routine extraction procedure is as follows: 100 gm. of the ground fish sample is homogenized in a Waring Blendor with 400 ml. of 1/10 *N* hydrochloric acid. A portion (50 gm.) of the homogenized material is autoclaved with approximately 100 ml. of 1/10 *N* hydrochloric acid at 15 lb. pressure for 15 min. The material is cooled to room temperature, the pH brought to between 4.5 and 5.0 with normal sodium hydroxide, and diluted to 200 ml. After centrifuging at 1200 r.p.m. for 15 min. the supernatant is poured off. If this extract is not perfectly clear, it must be filtered. Finally, a 50 ml. aliquot is adjusted to pH 6.8 and diluted to a suitable volume for assay at levels of 2, 3, and 4 ml. per culture tube.

Assay Procedure

The microbiological method of Snell and Strong has been slightly modified in that a vitamin supplement is added to the basal medium recommended in their original paper. In our experience, the growth curve is more regular when this supplement is added. A stock solution of the vitamins is prepared monthly and has the following composition: thiamin, 20 mgm.; nicotinic acid, 20 mgm.; pantothenic acid, 10 mgm.; pyridoxin, 20 mgm.; biotin, 10 ml. (*SMA* concentrate No. 200 containing 20 μgm. per ml.); water to a total

volume of 100 ml. One millilitre of this supplement is used for each 50 ml. of basal medium.

The inoculum is always prepared from an agar stab rather than from inoculum tubes as recommended by Snell and Strong (7). Assays are done in duplicate on duplicate extracts at levels of 2, 3, and 4 ml. of extract per tube. An average is taken of the values obtained if these do not differ by more than 10%. Dilutions are such that a culture tube with 10 ml. of fluid will contain 0.04 to 0.16 μgm. of riboflavin. Results of others have indicated that the method is less reliable beyond these limits (8).

Discussion of the Method as Applied to Fish

It has been shown repeatedly that *Lactobacillus casei*, grown in the broth recommended by Snell and Strong, may be stimulated by factors other than riboflavin. Studies on cereal products indicate that fatty acids are responsible (2, 8, 10). Inorganic constituents and unknown growth factors may also affect the results (4, 10). This stimulation is particularly evident when minimal amounts—less than 0.05 μgm.—of riboflavin are present (8). Hence the recovery of riboflavin added in minimal amounts to any particular food is commonly considered an index of the reliability of the method. Riboflavin may be added to the material being tested or the riboflavin in the extract may be entirely destroyed and a definite amount of the vitamin then added and recovered from this riboflavin-free extract. Recoveries of 90 to 110% of the added vitamin are considered satisfactory.

In the present studies the first method of checking was more frequently employed. In practice 20 μgm. of riboflavin is added to the homogenized fish prior to autoclaving, and the volumes are adjusted so that 1 ml. of the extract contains 0.025 μgm. of added riboflavin, and the total riboflavin content of the assay tube is somewhere between 0.04 and 0.16 μgm. Table I indicates that reliable results have been obtained in most cases.

In the second type of recovery experiment, the original riboflavin in the extracts is destroyed by alkaline photolysis (7, 10). Some of the photolysed extracts are then treated with ether to remove the fatty acids (10). In this case the assay tubes contain 5 ml. of basal medium, 4 ml. of photolysed extract, and 1 ml. of a standard riboflavin solution containing 0.05 μgm. of riboflavin. Maximal stimulation of growth by factors other than riboflavin might be expected at this level (8). Recoveries in this case (Table II) are higher than those obtained with the first procedure (Table I); as much as 10 to 40% more riboflavin was recovered than was added. Ether extraction improves the results but does not alter the general picture. Factors other than fatty acids seem to be responsible, This may be additional evidence for the unreliability of the growth curve at levels of 0.05 μgm. or less of riboflavin. It seems probable, however, that the alkaline photolysis, involving as it does the addition of large amounts of sodium hydroxide and hydrochloric acid, produces factors responsible for this stimulation. Chattaway *et al.* (4) find that the concentration of salts such as sodium chloride affect the assay.

TABLE I

RIBOFLAVIN CONTENT OF FISH TISSUES

Description	Reference No.	Moisture, %	Riboflavin, μgm. per gm.		Recovery of added riboflavin, %
			Fresh	Dry	
Clam (*Mya* sp.)					
Live specimens from St. Andrews, N.B., March	64	90.9	0.95	10.4	96.6
Canned 'Mephisto'	9	87.7	0.96	7.8	
'Mephisto'	13	86.9	0.93	7.2	90.0
'Mephisto'	17	86.5	0.92	6.8	107.0
'Eagle'	31	87.4	0.98	7.8	93.9
Cod (*Gadus callarias* Linnaeus)					
Frozen, Halifax, N.S., December					
muscle	24	82.3	1.00	5.6	96.6
skin	24	64.7	5.30	15.1	
liver	24	34.9	6.40	9.8	
Canned muscle, 'Maisonnette'	45	77.7	0.75	3.4	
Dried Salt, muscle	36	44.5	0.37	0.7	89.0
Eel (*Anguilla rostrata* Le Sueur)					
Frozen, Halifax, N.S., January					
muscle, 60.0 cm. fish	26 *A*	65.3	3.90	11.2	99.7
muscle, 45.0 cm. fish	26 *B*	65.3	5.20	15.0	
skin, combined	26 *A,B*	49.8	14.88	29.6	
Gaspereau (*Pomolobus pseudoharengus* Wilson)					
Canned skin and muscle, Fred Magee, Ltd.	22	78.0	1.77	8.0	100.5
Haddock (*Melanogrammus aeglifinus* Linnaeus)					
Frozen, Halifax, N.S., December					
muscle	27	80.7	0.57	2.9	100.0
skin	27	77.7	8.40	37.6	
liver	27	39.0	5.10	8.4	
Canned, Chicken Haddie,					
'Indian Chief'	4	75.5	0.50	2.0	
'Indian Chief'	8	74.5	0.70	2.7	
'Indian Chief'	53	74.7	0.35	1.4	
'Sea-Lect'	2	76.5	1.16	4.9	
'Sea-Lect'	47	78·0	0·48	2·2	
'Mephisto'	48	75.9	0.58	2.4	
'Mephisto'	52	79.4	0.42	2.0	
'Bluewater'	62	78.4	0.47	2.2	
Sea-sealed fillets (haddock?)					
Shipped in dry ice from Halifax—October	15	81.5	0.95	5.1	82.5
Local purchase—March	54	82.4	0.55	3.1	
Chilled muscle, local purchase	55	82.7	0.88	5.1	
	59	83.0	1.05	6.2	
	68	83.8	0.55	3.4	
Muscle, sample No. 59, salted for six days	59 *A*	61.6	1.30	3.4	
Finnan Haddie, local purchase	44	78.2	1.03	4.7	89.1
	67	81.1	0.70	3.7	
Finnan Haddie, Canned, 'Brunswick'	23	74.8	0.55	2.2	85.3
Halibut (*Hippoglossus hippoglossus* Linnaeus)					
Chilled muscle, local purchase	56	76.8	0.55	2.4	90.2
	69	79.6	0.68	3.3	
Frozen muscle, local purchase	65	75.3	0.80	3.2	
Muscle, sample No. 65, salted for eight days	65 *A*	55.8	1.40	3.2	
Herring (*Clupea harengus* Linnaeus)					
Frozen, St. Andrews, N.B., February					
14 to 18 cm., muscle	40	78.7	1.50	7.1	106.0
skin	40	72.7	0.25	0.9	
8 to 10 cm., muscle	41	78.8	1.50	7.1	
skin	41	70.9	0.35	1.2	
Canned, 'Bay of Fundy', muscle and skin	28	75.0	2.00	8.0	95.5
Spiced, 'Sea Nymph'	35	73.8	1.70	6.7	89.2
Sardines in oil, 'Glacier'	29	49.3	2.10	4.1	92.7
Salted, 35 cm., local purchase					
muscle	50	49.4	1.90	3.8	100.0
skin	50	42.3	4.90	8.5	
Kippered, 20.0 cm., local purchase,					
muscle	38	56.3	3.10	7.1	93.4
skin	38	36.3	4.50	7.1	
Kippered, dried, 12.0 cm., local purchase,					
muscle	39	36.9	4.50	7.1	

TABLE I—*Concluded*

RIBOFLAVIN CONTENT OF FISH TISSUES—*Concluded*

Description	Reference No.	Moisture, %	Riboflavin, μgm per gm.		Recovery of added riboflavin, %
			Fresh	Dry	
Lobster (*Homarus americanus* Milne-Edwards)					
Live, 30.0 cm. specimen from St. Andrews, July					
muscle	72	81.2	0.55	2.9	
Canned, muscle, 'Eagle'	12	77.9	0.75	3.4	
'Eagle'	16	77.6	0.72	3.2	74.0
'Mephisto'	66	77.6	0.58	2.6	95.0
Canned paste, 'P.E.I. Co.'	34	71.3	2.80	9.8	104.0
'P.E.I. Co.'	49	72.3	2.40	8.7	97.7
Mackerel (*Scomber scombrus* Linnaeus)					
Frozen, Halifax, N.S., November					
muscle	20	58.1	2.80	6.7	86.5
skin	20	62.2	4.85	12.8	
liver	20	34.1	7.8	11.8	
Canned, muscle and skin					
'East Coast'	14	64.9	2.1	6.0	89.1
'East Coast'	70	58.8	2.7	6.6	
Mussel (*Mytilus edulis* Linnaeus)					
Live specimens from St. Andrews, N.B., March	63	90.4	1.75	18.2	94.7
Canned, 'Sea-Lect'	30	86.6	1.38	10.3	99.0
'Fraser'	60	87.9	1.15	9.5	
Oyster (*Ostrea virginica* Gmelin)					
Live specimens from Ellerslie, P.E.I., July	74	85.9	2.10	14.9	
Pollock (*Pollachius virens* Linnaeus)					
Frozen, Halifax, N.S., December					
muscle	25	80.7	1.23	6.4	89.8
skin	25	73.9	6.00	23.0	
liver	25	34.3	4.60	7.0	
roe	25	73.8	3.45	13.2	
Quahaug (*Venus mercenaria* Linnaeus)					
Live specimens, from Ellerslie, P.E.I., July	73	92.7	1.30	17.8	
Canned, 'Neguac Bay'	42	83.0	0.95	5.6	91.5
'Under Two Flags'	43	82.2	1.70	9.6	
'Red Snapper'	61	85.1	1.13	7.6	
Scallop (*Pecton* sp.)					
Canned, 'Beaton's'	37	85.6	0.70	4.8	94.4
Salmon, Miramichi (*Salmo salar* Linnaeus)					
Local purchase, July					
muscle	75	67.2	0.60	1.8	
skin	75	53.3	1.75	3.7	
Shad (*Alosa sapidissima* Wilson)					
Local purchase, May					
muscle	71	71.0	2.40	8.3	
skin	71	55.2	3.70	8.3	
Muscle, sample No. 71, salted for two months	71 *A*	66.5	1.40	4.2	
Canned muscle and skin, Fred Magee, Ltd.	21	58.1	1.85	4.4	96.0
Canned muscle and skin, Fred Magee, Ltd.	46	62.7	1.33	3.6	
Smelt (*Osmerus mordax* Mitchill)					
Frozen, Loggieville, N.B., January					
muscle	32	79.5	1.28	6.2	89.9
skin	32	68.1	1.40	4.4	
Sole (*Pseudopleuronectes americanus* Walbaum)					
Frozen, Halifax, N.S., March					
muscle	51	82.9	0.70	4.1	101.3
muscle	51 *A*	79.1	0.65	3.1	
skin	51	67.0	3.35	10.1	97.3
roe	51	70.7	4.30	14.7	
Tomcod (*Microgadus tomcod* Walbaum)					
Frozen, Loggieville, N.B., January					
muscle	33	81.3	1.70	9.1	95.2
skin	33	78.7	4.20	19.7	
Trout (*Salvelinus fontinalis* Mitchill)					
Shipped from St. John in ice, July, 30.0 to 35.0 cm. specimens, Howe Lake					
brilliant pink muscle	76	74.0	0.65	2.5	
skin	76	62.4	1.35	3.6	
15.0 to 20.0 cm. specimens, McDowell Brook					
white muscle	77	75.3	0.80	3.2	

As indicated above, an ether extraction of the photolysed extracts brought about a definite improvement in the percentage of added vitamin recovered (Table II). The advisability of treating all extracts with ether was, therefore, considered. Since, however, the lowering produced by this procedure (Tables II and III) is well within the error of 10% usually considered inherent in the microbiological method, and since the labour of ether extraction is considerable, the procedure was not adopted routinely.

TABLE II

RECOVERY OF RIBOFLAVIN ADDED TO PHOTOLYSED EXTRACTS OF FISH TISSUES

Material	Reference No.	Vitamin recovered, %	
		Photolysed extract	Ether treated photolysed extract
Clam	13	120.0	106.0
Gaspereau, canned	22	120.0	109.0
Haddock, fillets	15	120.0	114.0
Finnan Haddie	23	110.0	
Herring, canned	28	118.0	106.0
Mussels, canned	30	106.0	103.0
Shad, canned	21	130.0	120.0
Shad, canned	46	137.0	119.0

TABLE III

THE EFFECT OF ETHER EXTRACTION ON THE RIBOFLAVIN VALUES OF FISH EXTRACTS

Fish	Sample No.	Riboflavin, μgm. per 100 gm. tissue		
		Untreated extract	Ether-treated extract	Lowering, %
Clams, canned	17	95	90	5.3
Clams, canned	31	98	93	5.1
Cod, muscle	24	100	93	7.0
Cod, skin	24	530	500	5.9
Mackerel, muscle	20	280	270	3.6

Results

Distribution in Different Tissues

Table I shows that the different tissues of fish vary greatly in their riboflavin content. In general, it appears that skin has a high and muscle a low riboflavin value. Liver and roe are intermediate between skin and muscle. The skin of cod, eel, mackerel, pollock, salmon, sole, and tomcod was found to contain from two to four times as much riboflavin per gram of dry tissue as the muscle. The skin of small herring and smelt were found to have extremely low values. It is felt, however, that in these cases little more than the epidermis of the skin was obtained for assay. It is probable that the vitamin

is concentrated in the deeper layers of the skin or even the superficial layer of muscle that frequently adheres to the skin when the latter is removed.

The glandular tissues of animals are known to be richer in riboflavin than muscle but no one seems to have compared skin with muscle. This comparatively high value for fish skin may account for some of the variability in results obtained by workers who did not assay these tissues separately.

Size of Animal and Riboflavin Content

The University of Texas workers have studied riboflavin in a great many different animal species. They conclude that there is an inverse ratio between the size of the organism and the B-vitamin content of its tissues (11). This generalization holds for different sized individuals of a class—e.g., man, rat, mouse in mammals—or for individuals of different phyla—e.g., rat, clam, fruit fly, etc. Our findings (Table IV) do not indicate that any such relationship exists for fish.

TABLE IV

ANIMAL SIZE AND THE RIBOFLAVIN CONTENT

Fish	Reference No.	Approx. wt., kgm.	Riboflavin, μgm. per gm., dry	
			Muscle	Skin
Pollock	25	5.5	6.4	23.0
Cod	24	3.5	5.6	15.1
Haddock	27	2.0	2.9	37.6
Shad	71	2.0	8.3	8.3
Sole	51	1.5	3.5	10.1
Mackerel	20	0.5	6.7	12.8
Tomcod	33	0.25	9.1	19.7
Eel	26*b*	0.25	11.2	
Trout	76	0.25	2.5	3.6
Eel	26*a*	0.15	15.0	
Trout	77	0.05	3.2	
Smelt	32	0.04	6.2	4.4
Herring	40	0.03	7.1	0.9
Herring	41	0.01	7.1	1.2

The Texas group do not seem to have compared the vitamin content of tissues from different sized individuals of the same genus or species. We have done this for the eel and the herring. A difference was evident in the eel samples but no difference has been demonstrated for either fresh or kippered herring (Tables I and IV). It is not felt that the difference in the case of eel is significant, since two fish of the same size and the same species may vary somewhat in their vitamin content. Samples No. 51 and No. 51*A* (Table I) were from two different flounders of the same size taken at the same time and treated in the same manner. One contained 3.1 and the other 4.1 μgm. per gram of dry muscle. We conclude that there is no relationship between size and the riboflavin content of the tissues.

Processing

Freezing

Samples of beef, pork, finnan haddie, haddock, halibut, and shad were frozen at the New Brunswick Apple Exchange for varying periods up to eight weeks. Although three out of four of the values are lower than the original values, all are within the range of error involved in this method (Fig. 1). It is concluded, therefore, that losses of riboflavin brought about by fast freezing are negligible.

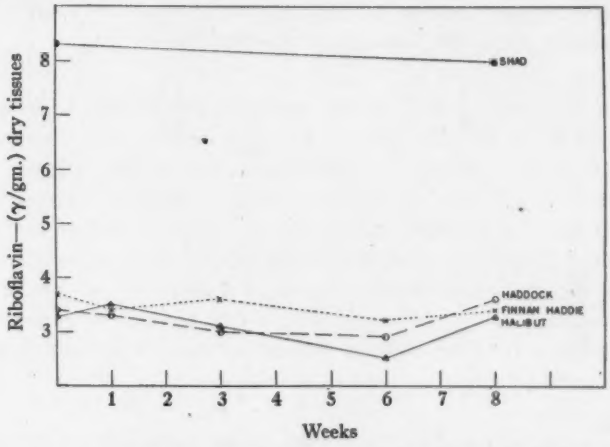

FIG. 1. *The effect of freezing on the riboflavin content of fish muscle.*

Canning

In a recent summary of the data available on the B-vitamins in fish, Lovern (6) concludes that canning does not affect the riboflavin content greatly. We find, on the other hand, that canned products are extremely variable in riboflavin content (Table I). In a series of Chicken Haddie samples, for example, the values ranged from 1.4 to 4.9 μgm. per gm. of dry tissue. This may be due to differences in the material canned or it may indicate that the canning procedures destroy variable amounts of riboflavin.

As already stated the riboflavin content of fresh fish of any particular variety is variable. Thus, in fresh haddock the values ranged from 2.9 to 6.9 μgm. per gm. of dry muscle. Eel, flounder, and halibut showed variations of like order. The values for trout muscle are interesting in this regard. The muscle of sample No. 76 was bright pink in colour, while that of No. 77 was almost white. Such differences in colour are usually explained on the basis of diet. The muscle of sample No. 76 contained 2.5 μgm. and that of No. 77 contained 3.2 μgm. of riboflavin per gm. Thus, a certain variation in the canned products will be related to the differences found in the fresh fish.

The factors responsible for these variations must be investigated if canned fish of the highest possible riboflavin content is to be produced.

However, it would appear that the canning methods employed in the Maritimes do result in the destruction of appreciable amounts of riboflavin. The values for canned products are consistently lower than those for the fresh fish. This is particularly evident in the case of the shellfish (Table I).

Smoking

Smoking produces little change in riboflavin content (Table I). Values for finnan haddie and for kippered herring are close to those for fresh fish. This is in agreement with the findings of Lovern (6).

Salting

In many cases the salting of fish produces definite losses in riboflavin (Table I). This is probably related to the withdrawal of water from the tissues. Salt herring purchased locally contained 3.8 μgm. whereas the fresh fish contained 7.1 μgm. per gram of dry muscle. In one experiment, haddock, halibut, and shad were salted for 6, 8, and 60 days, respectively. Fine powdered sodium chloride was rubbed into the muscle, the fish packed in bottles and stored at room temperature. Losses of about 50% of the vitamin in haddock and shad are of the same order as the losses indicated in the salt herring purchased locally. Halibut showed no loss. The salt concentration is probably important.

Drying

Methods of drying vary greatly. Where the fish are dried in sunlight a destruction of the vitamin is to be expected. In a sample of dried salt cod (No. 36, Table I), the riboflavin had almost entirely disappeared. In the dried kippered herring (No. 39, Table I) it was present in normal amounts. It is possible to dry meat without loss of the riboflavin (9) and the same is probably true for fish.

Fish as a Source of Riboflavin

The data of Waisman and Elvehjem (9) for meat have been used in the following comparisons of the relative values of meat and fish. On the whole, it appears that fish provides less riboflavin per gram than meat (Fig. 2). The marine forms most frequently eaten—cod, haddock, halibut, mackerel, herring—contain less than 60% of the riboflavin found in beef, lamb, pork, or dark poultry meat. The University of Texas workers (11) report similar differences. Fish skin contains more riboflavin than mammalian muscle (Table I) but in the quantities eaten would not provide the extra riboflavin necessary to make meat and fish of equal nutritive value. Fish liver, although much higher than fish muscle, contains only about 1/10th the riboflavin found in mammalian liver or kidney. Skin and roe have a higher riboflavin content than liver, but are, nevertheless, lower than mammalian glands.

ERRATUM

In the block for "POULTRY" in the cut for Fig. 2, the words "LIGHT" and "DARK" should be transposed.

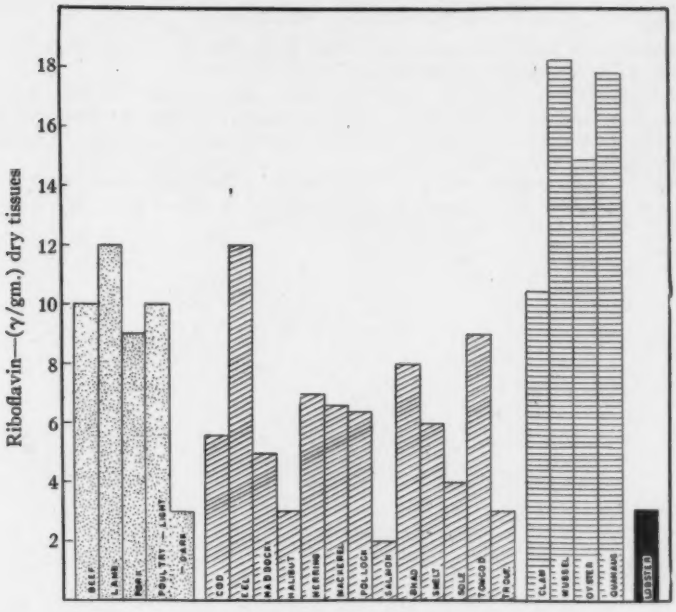

FIG. 2. *Riboflavin in meat and fish.*

Molluscan tissues are much richer than either fish or mammalian muscle (Fig. 2). They do not, however, compare with mammalian liver or kidney as concentrated sources of riboflavin. Waisman and Elvehjem (9) give values of from 85 to 100 μgm. per gm. of dry mammalian glandular tissue. These figures are 5 to 10 times greater than those obtained for molluscan tissues.

Values obtained for lobster muscle are of the same order as the lowest of the fish values—haddock, halibut, salmon, and trout (Table I, Fig. 2). Analyses of canned lobster paste indicates that the glands of the lobster contain about three times as much riboflavin as the muscle.

A diet that consists largely of fish will be lower in riboflavin than diets containing an equivalent amount of meat. Waisman and Elvehjem (9) have calculated that, for the middle class American wage earner, meat provides 25% to 30% of the riboflavin requirement.

References

1. ANDREWS, J. S. Cereal Chem. 20 : 3-25. 1943.
2. BAUERNFIEND, J. C., SOTIER, A. L., AND BORUFF, C. S. Ind. Eng. Chem., Anal. Ed. 14 : 666-671. 1942.
3. BILLINGS, F. L., BIELY, J., FISHER, H., and HEDREEN, C. J. Nutrition. 22 : 425-430. 1941.

4. CHATTAWAY, F. W., HAPPOLD, F. C., and SANDFORD, M. Biochem. J. 37 : 298-302. 1943.
5. CHELDELIN, V. H. and WILLIAMS, R. J. Univ. Texas Pub. No. 4237 : 105-124. 1942.
6. LOVERN, J. A. Chemistry & Industry, 62 : 328-330. 1943.
7. SNELL, E. E. and STRONG, F. M. Ind. Eng. Chem., Anal. Ed. 11 : 346-350. 1939.
8. STRONG, F. M. and CARPENTER, L. E. Ind. Eng. Chem., Anal. Ed. 14 : 909-913. 1942.
9. WAISMAN, H. A. and ELVEHJEM, C. A. The vitamin content of meat. Burgess Publishing Co., Minneapolis. 1941.
10. WEGNER, M. I., KEMMERER, A. R., and FRAPS, G. S. J. Biol. Chem. 144 : 731-735. 1942.
11. WOODS, A. M., TAYLOR, J., HOFER, M. J., JOHNSON, G. A., LANE, R. L., and MCMAHAN, J.R. Univ. Texas Pub. No. 4237 : 84-86. 1942.

CANADIAN JOURNAL OF RESEARCH

Notes on the Preparation of Copy

General:—Manuscripts should be typewritten, double spaced, and the **original and at least one extra copy** submitted. Style, arrangement, spelling, and abbreviations should conform to the usage of this Journal. Names of all simple compounds, rather than their formulae, should be used in the text. Greek letters or unusual signs should be written plainly or explained by marginal notes. Superscripts and subscripts must be legible and carefully placed. Manuscripts should be carefully checked before being submitted, to reduce the need for changes after the type has been set. **All pages, whether text, figures, or tables, should be numbered.**

Abstract:—**An abstract of not more than about 200 words, indicating the scope of the work and the principal findings, is required.**

Illustrations

(i) *Line Drawings:*—Drawings should be carefully made with India ink on white drawing paper, blue tracing linen, or co-ordinate paper ruled in **blue** only. Paper ruled in green, yellow, or red should not be used. The principal co-ordinate lines should be ruled in India ink and all lines should be of sufficient thickness to reproduce well. Lettering and numerals should be of such size that they will not be less than one millimetre in height when reproduced in a cut three inches wide. If means for neat lettering are not available, lettering should be indicated in pencil only. All experimental points should be carefully drawn with instruments. Illustrations need not be more than two or three times the size of the desired reproduction, but the ratio of height to width should conform with that of the type page. **The original drawings and one set of small but clear photographic copies are to be submitted.**

(ii) *Photographs:*—Prints should be made on glossy paper, with strong contrasts; they should be trimmed to remove all extraneous material so that essential features only are shown. Photographs should be submitted **in duplicate**; if they are to be reproduced in groups, one set should be so arranged and mounted on cardboard with rubber cement; the duplicate set should be unmounted.

(iii) *General:*—**The author's name, title of paper, and figure number should be written on the back of each illustration.** Captions should not be written on the illustrations, but typed on a separate page of the manuscript. All figures (including each figure of the plates) should be numbered consecutively from 1 up (arabic numerals). **Reference to each figure should be made in the text.**

Tables:—Titles should be given for all tables, which should be numbered in Roman numerals. Column heads should be brief and textual matter in tables confined to a minimum. **Reference to each table should be made in the text.**

References should be listed alphabetically by authors' names, numbered in that order, and placed at the end of the paper. The form of literature citation should be that used in this Journal and titles of papers should not be given. All citations should be checked with the original articles. Each citation should be referred to in the text by means of the key number.

The *Canadian Journal of Research* conforms in general with the practice outlined in the *Canadian Government Editorial Style Manual*, published by the Department of Public Printing and Stationery, Ottawa.

Reprints

Fifty reprints of each paper are supplied free. Additional reprints, if required, will be supplied according to a prescribed schedule of charges.